Norio SAHASHI*: Morphological and taxonomical studies on Ophioglossales in Japan and the adjacent regions (2)**

Comparative morphology of spores in the genus *Botrychium* s. str.

佐橋紀男*: 日本および近隣産ハナヤスリ目の形態学的分類学的研究 (2)**

ヒメハナワラビ属胞子の形態の比較

(Pl. I—II)

The spore morphology of the genus *Botrychium* Swartz s. str. (Nishida, 1952) or subgenus Eubotrychium Clausen (1938) was studied both under the light (LM) and scanning electron microscope (SEM).

Materials and results

1a. **Botrychium boreale** Milde ssp. **boreale**: Mt. Paiktusan, Korea, Jul. 1931. T. Ishidoya (KYO); Mt. Usuzan, Hokkaido, Aug. 1976. N. Sahashi (TOHO); Finland (UC, 1363426).

For details see the author's previous report (1978).

1b. **B. boreale** ssp. **obtusilobum** (Ruprecht) Clausen: Minnesota, U. S. A. (UC, 955467); Smithers, Canada (UC, M̄305894).

Shape: Trilete. Amb subtriangle (ST) to often rounded triangle (RT) or triquete (TQ), proximal part (PP) flatly pyramidal to convex, distal part (DP) hemispherical or often subconical. *Size*: 35(30-40)×40(36-43)μ. *Laesura*: 15-21μ long, somewhat prominent, up to 2.5μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2μ thick, with partially irregular verrucae up to 1.5μ high and 2-5μ in diameter on the distal face (DF), almost subpsilate on the proximal face (PF). At high magnification under SEM, some rough projections can be seen on PF, and fine granules are scattered all over the surface. DF more minutely sculptured than in ssp. *boreale* (Pl. II. Fig. 1. K, L).

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** Continued from Journ. Jap. Bot. 53: 51-60. 1978.

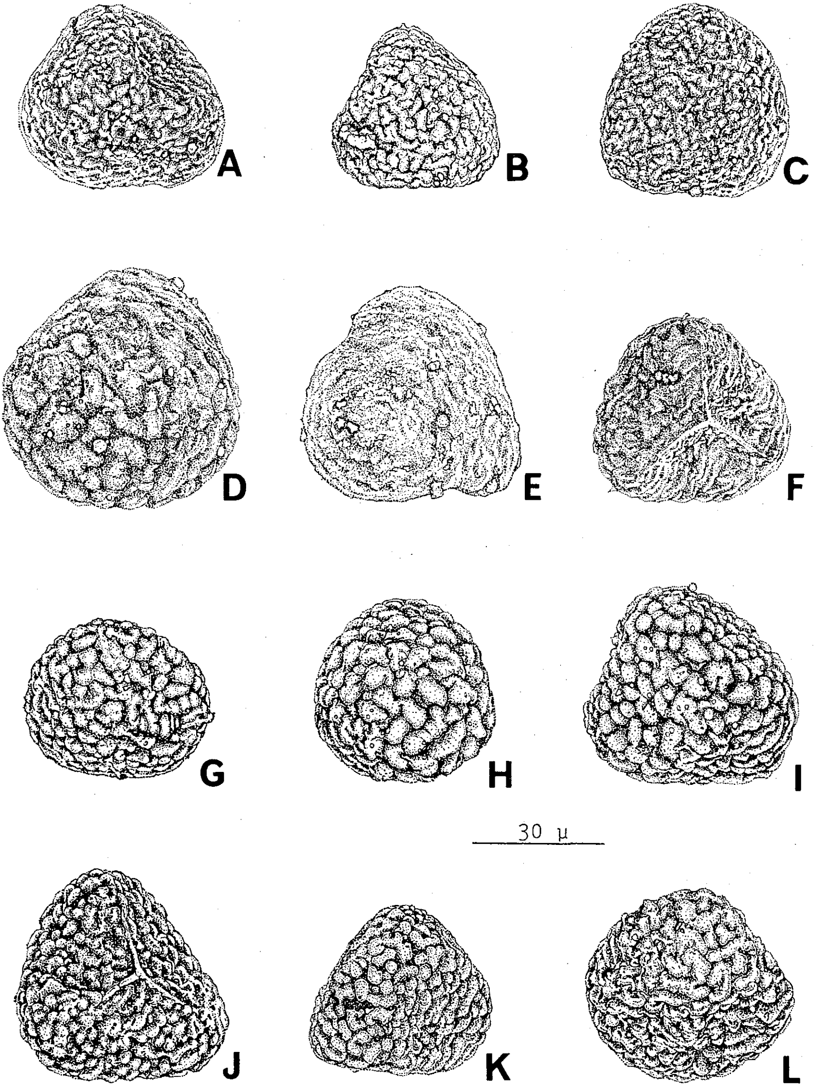

Fig. 1. Spores illustrated from LM and SEM observations. A, B. *Botrychium pumicola*. A. Polar view, distal face, from Oregon (UC, 955464). B. Polar view, distal face, from Oregon C. *B. simplex* var. *tenebrosum*, polar view, distal face, from Canada (TNS, 163052). D—F. *B. simplex* var. *simplex*. D. Polar view, distal face, from California (TOHO). E. Polar view, distal face. F. Polar view, proximal face. E, F. From Finland (UC, 250772). G-J *B. boreale* ssp. *boreale*. G. Polar view, distal face, from Korea (KYO). H. Polar view, distal face, from Hokkaido (TOHO). I. Polar view, distal face. J. Polar view, proximal face. I, J. From Finland (UC, 1363426). K, L. *B. boreale* ssp. *obtusilobum*. K. Polar view, distal face, from Canada (UC, M 305894). L. Polar view, distal face, from U.S.A. (UC, 955467).

2a. **B. lanceolatum** (Gmel.) Angstr. ssp. **lanceolatum**: Mt. Rishirisan, Hokkaido, Aug. 1903. T. Makino (MAK); Hamatonbetsu, Hokkaido, Jul. 1977. M. Hara (TOHO)[1]; Utah, U.S.A. (UC, 943302); Washington, U.S.A. (UC, 810894).

Spores of this species were described in detail in the previous paper (1976). In the present study, therefore, those of materials collected outside of Japan are reported. *Shape*: Trilete. Amb ST or often TQ, PP flatly pyramidal to often convex, DP hemispherical or subconical. *Size*: 30(25-35) × 36(30-40) μ. *Laesura*: 12-20 μ long, somewhat prominent, up to 4 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2 μ thick, DF densely tuberose or often irregularly extervermiculate, tubers up to 2 μ high and 3-6 μ in diameter, PF covered with small verruca-like projections by SEM. In Japanese specimens, spores are minutely pitted or punctate on PF, but material from Washihgton has small verruca-like projections (Pl. II. I; Fig. 2. U, V, W).

2b. **B. lanceolatum** ssp. **angustisegmentum** (Pease et Moore) Clausen: New York, U.S.A., Aug. 1920. W.C. Muenscher, et al. (TI); N. America (UC, 199992).

Shape: Trilete. Amb ST or often TQ, PP flatly pyramidal, DP hemispherical or sometimes subconical. *Size*: 27(24-30) × 33(30-35) μ. *Laesura*: 10-18 μ long, slightly prominent, up to 2 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 1.5 μ thick, DF somewhat irregularly tuberose, tubers up to 1.5 μ high and 2-5 μ in diameter, PF covered with small verruca-like projections by SEM. The spores are smaller than those of ssp. *lanceolatum*, but the ornamentations on DF are very similar to those of Japanese ssp. *laneolatum* rather than to those of foreign ones (Pl. II. J; Fig. 2. X).

3a. **B. lunaria** (L.) Sw. var. **lunaria**: Toyako, Hokkaido, Aug. 1976. N. Sahashi (TOHO); Mt. Tekaridake, Nagano Pref., cultivated in Tokyo, Jul. 1977. T. Mishina (TOHO); Mt. Yatsugatake, Nagano Pref., Aug. 1922. T. Sato (MAK, 178. TNS, 142297); Chickaloon, Alaska, Jul. 1975. K. Moriya (private herbarium of Dr. K. Moriya).

Details are quite similar both in the materials concerned in author's previous paper (1976) and the Japanese ones cited above. Details obtained from

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1). This is the first record of the subspecies for Hokkaido proper.

the Alaskan material are as follows. *Shape*: Trilete. Amb RT to often ST, PP flatly pyramidal to convex, DP hemispherical to often heavily arched. *Size*: 38(34-42)×45(41-47) μ. Spores are larger than those of Japanese materials which were shown in the previous paper (1976). *Laesura*: 17-25 μ long, clear prominent, c. 3 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, up to 2.5 μ thick with projections, DF verrucose or often anastomosing to extervermiculate, verrucae up to 1 μ high and 2-5 μ in diameter, PF covered with smaller projections than DF and faintly punctate on the contact area by SEM. The specimen from Mt. Yatsugatake reported under *B. simplex* var. *tenebrosum* by Nishida in Acta Phytotax. Geobot. **18**: 39. 1959 can be identified with this species. In spores of this specimen under SEM study, the ornamentations on DF are irregularly verrucose, and often their prominently undulated ridges appear to be extervermiculate. On PF, the smaller verrucae than on DF are more or less sparsely scattered, or often subpsilate (Pl. I. C, D; Pl. II. K, L; Fig. 2. M, N, O).

3b. **B. lunaria** var. **minganense** (Victorin) Dole: Graham Isl., Canada (UC, 1333117); Michigan, U.S.A. W.H. Wagner (KYO).

Shape: Trilete. Amb ST to often TQ, PP flatly pyramidal to convex, DP hemispherical or often subconical. *Size*: 32(29-35)×40(37-44) μ. *Laesura*: 17-24 μ long, clearly prominent, up to 3 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2 μ thick with projections, DF verrucose or partially extervermiculate, verrucae less than 1 μ high and 3-6 μ in diameter, PF subpsilate by LM, with verruca-like small projections by SEM. The spores in material from Michigan contain somewhat immature spores. The immature spores have minute granules on DF by SEM (Pl. II. M; Fig. 2. P, Q).

4, **B. matricariaefolium** Al. Braun: Michigan, U.S.A. Jun. 1957. W.H. Wagner (KYO); New Hampshire, U.S.A. Jun. 1937. A.R. Hodgdon (TI); Trieben, Austria, Jul. 1909. E. Knek (KYO).

Shape: Trilete. Amb ST to often TQ, PP flatly pyramidal to convex, DP hemispherical or sometimes subconical. *Size*: 30(27-33)×36(34.5-38) μ. *Laesura*: 15-20 μ long, somewhat prominent, up to 3 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2 μ thick including sculptures, DF minutely verrucose, verrucae less than 1 μ high and 2-4 μ in diameter, PF covered with minute granules. The exine of the present species is more clearly verrucose than in any other taxon treated in this study (Pl. II. N;

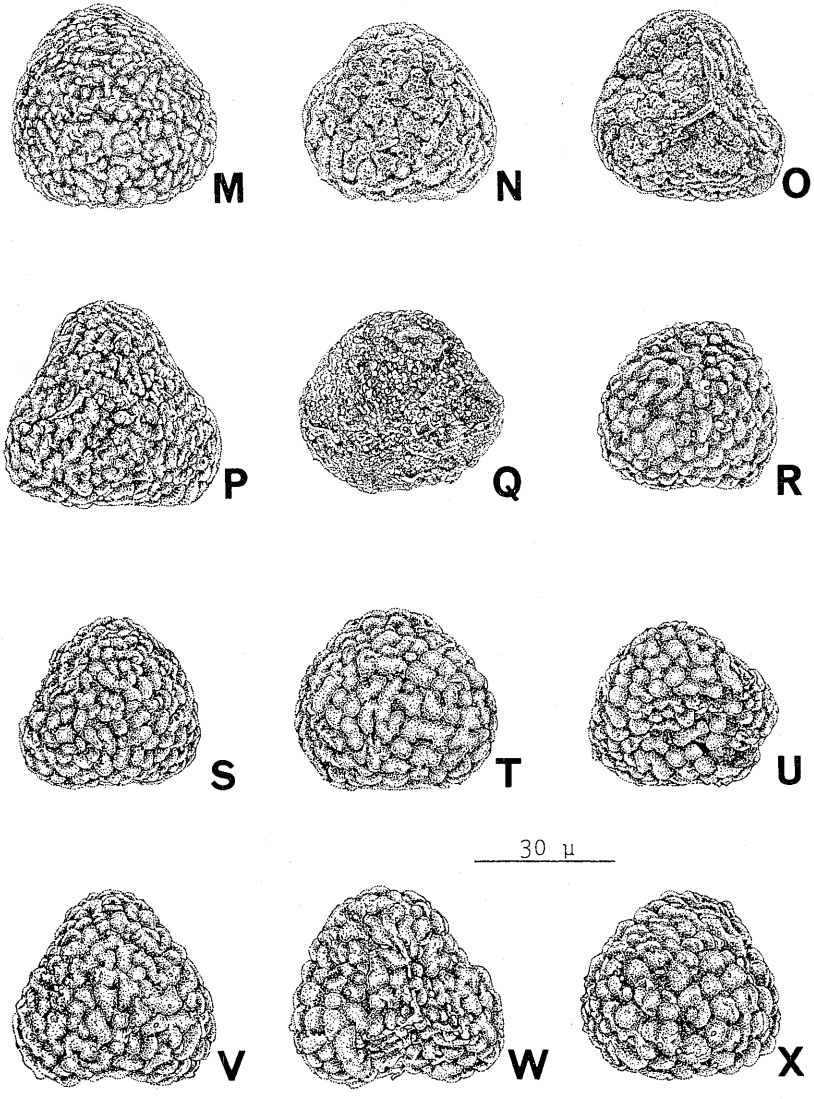

Fig. 2. M-O. *B. lunaria* var. *lunaria*. M. Polar view, distal face, from Alaska (private herbarium of Dr. K. Moriya). N. Polar view, distal face. O. Polar view, proximal face. N, O. From Hokkaido (TOHO). P, Q. *B. lunaria* var. *minganense*. P. Polar view, distal face, from Canada (UC, 1333117). O. Polar view, distal face, from Michigan (KYO). R-T. *B. matricariaefolium*. R. Polar view, distal face, from New Hampshire (TI). S. Polar view, distal face, from Michigan (KYO). T. Polar view, distal face, from Europe (as *B. ramosum* in KYO). U-W. *B. lanceolatum* ssp. *lanceolatum*. U. Polar view, distal face, from Hokkaido (MAM). V. Polar view, distal face. W. Polar view, proximal face. V, W. From Utah (UC, 943302). X. *B. lanceolatum* ssp. *angustisegmentum*, polar view, distal face, from New York (TI).

Fig. 2. R, S, T).

5. **B. pumicola** Coville: Oregon, U. S. A. (UC, 955464); Oregon, U. S. A., Aug. 1963. L. Kiefer (TOHO).

Shape: Trilete. Amb ST or often TQ, PP flatly pyramidal to often convex, DP hemispherical or heavily arched. *Size*: 30(27-33)×36(33.5-40) μ. *Laesura*: 12-19 μ long, somewhat prominent, up to 3 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2 μ thick including sculptures, DF verrucose, verrucae condense, less than 1 μ high and up to 3 μ in diameter, PF subpsilate by LM, faintly granular by SEM (Pl. II. O; Fig. 1. A, B).

6a. **B. simplex** Hitchcock var. **simplex**: Finland (UC, 250772); Yosemite, Calif., Sept. 1963. L. Kiefer (TOHO).

Shape: Trilete. Amb RT to ST or often TQ, PP convex, DP hemispherical or heavily arched. *Size*: 39(34-45)×48(43-55) μ. *Laesura*: 21-30 μ long, clear prominent, up to 4 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2.5 μ thick with projections, DF faintly covered with verruca-like projections, verrucae less than 1 μ high and 3-5 μ in diameter, or often subpsilate, PF subpsilate or rarely punctate at the contact area by SEM, but faintly granular by LM. The spores of this variety have indistinct sculptures on DF and PF, but are larger in size than those of any other taxon treated in this study (Pl. I. A, B; Fig. 1. D, E, F).

6b. **B. simplex** var. **tenebrosum** (A. A. Eaton) Clausen: Bella Coola, Canada, Jul. 1956. (TNS, 163052).

Shape: Trilete. Amb RT to ST or often TQ, PP flatly pyramidal to convex, DP hemispherical, or often heavily arched. *Size*: 34(31-37)×41(38-43) μ. *Laesura*: 17-25 μ long, clearly prominent, up to 4 μ tall at the proximal pole. *Exine*: Light yellow, semitransparent, c. 2.5 μ thick including sculptures, DF densely covered with verruca-like projections, verrucae less than 1 μ high and 3-5 μ in diameter, PF nearly smooth or often faintly verrucose by LM, subpsilate or often more minutely verrucose than DF by SEM. The distal ornamentations of this variety have more clear sculpture than those of var. *simplex*. However, spores are a little smaller than those of var. *simplex* (Pl. I. E, F; Fig. 1. C).

Discussion

A palynological survey on the spores of all six species belonging to the subgenus Eubotrychium Clausen (1938) was carried out in the present study. The author (1976, 1978) already reported on spores of four species in Japan belonging to the genus *Botrychium* sensu Nishida (1952, 1959). From his previous investigations (1976, 1978) and present results, the author found that Nishida's *Botrychium* to be divided into three groups, while Clausen (1938) divided his subgenus Eubotrychium into two sections, Lunariae and Lanceolatae. The former includes five species and the latter one species.

The following is a key to the species on the basis of spore characters.

A. Spores 25-40×30-47 μ, exine clearly verrucose or often verrucose to extervermiculate ······································Sect. Lunariae (p. maj. p.)
B. Exine clearly verrucose ·······················*B. matricariaefolium*
BB. Exine somewhat large-verrucose to extervermiculate
····*B. lunaria* var. *lunaria*
····*B. boreale* ssp. *boreale*
BBB. Exine somewhat small-verrucose to extervermiculate
····*B. lunaria* var. *minganense*
····*B. boreale* ssp. *obtusilobum*
····*B. pumicola*
AA. Spores 30-45×38-55 μ, exine covered with verruca-like projections or often subpsilate································Sect. Lunariae (p. min. p.)
C. Exine faintly covered with verruca-like projections or often subpsilate, spores 39×48 μ in average ···················*B. simplex* var. *simplex*
CC. Exine covered with verruca-like projections, spores 34×41 μ in average···*B. simplex* var. *tenebrosum*
AAA. spores 25-35×30-40 μ, exine tuberose or rarely verrucose to extervermiculate. ···Sect. Lanceolatae
D. Spores 24-30×30-40 μ ················*B. lanceolatum* ssp. *lanceolatum*
DD. Spores 24-30×30-35 μ ··········*B. lanceolatum* ssp. *angustisegmentum*

Sect. Lanceolatae can be separated from Sect. Lunariae on the basis of the spore ornamentations, viz. the former are decorated with tubers and the latter with verrucae. Furthermore Sect. Lunariae can be divided into two groups, viz. Sect. Lunariae (p. maj. p.) and Sect. Lunariae (p. min. p.);

species of the former group producing small spores with clear sculptures and those of the latter larger spores with somewhat indistinct sculptures. Therefore, from a palynological point of view, a new section may be able to be established for Sect. Lunariae (p. min. p.) in the future.

Nakamura et al. (1957), Sladkov (1959) and Erdtman et al. (1971) reported larger spores than the present study. These may be derived from different spore treatment. Erdtman et al. (1961) suggested that the chemical treatment (acetolysis, according to Erdtman, 1952) sometimes affects the *Botrychium* spores more than other fern spores, and the spores give a great deal of swelling. However a little difference of spore size is found even by the same treatment in the same variety, for example in *B. lunaria* var. *lunaria* from Alaska and from Japan (Fig. 2. M, N, O), though spores of the variety from these two areas belong to the same group of large size. The difference seems to be derived from cytological conditions. But at least in the subgenus Eubotrychium Clausen, there is little report on the relationship between spore characters and cytological conditions such as suggested by Wagner & Lord (1956).

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Explanation of Plates I-II

Plate I. A, B. Spores of *Botrychium simplex* var. *simplex* from Finland (UC, M̄250772). A. Polar view, distal face. B. A part of distal face, showing somewhat rough small projections. C, D. *B. lunaria* var. *lunaria* from Mt. Yatsugatake, Nagano Pref. (TNS, 142297). C. Polar view, distal face. D. A part of distal face, showing verruca-like projections as in the spores of normal *B. lunaria* var. *lunaria*. E, F. *B. simplex* var. *tenebrosum* from Canada (TNS, 163052). E. Polar view, distal face. F. A part of distal face, showing smaller verruca-like projections than those of *B. lunaria* var. *lunaria*.

Plate II. G. *B. boreale* ssp. *boreale* from Mt. Usuzan, Hokkaido (TOHO), showing intermediate form between tubers and verrucae. H. *B. boreale* ssp. *obtusilobum* from Canada (UC, M̄305894), showing smaller ornamentations than those of ssp. *boreale*. I. *B. lanceolatum* ssp. *lanceolatum* from Utah, U. S. A. (UC, 943302), showing verruca-like projections. J. *B. lanceolatum* ssp. *angustisegmentum* from New York, U. S. A. (TI), showing somewhat irregular tubers. K, L. *B. lunaria* var. *lunaria*. K. From Hokkaido (TOHO), showing intermediate form between verrucae and extervermiculae. L. From Alaska (private herbarium of Dr. K. Moriya), showing a larger spore with smaller ornamentations than those of the previous specimen. M. *B. lunaria* var. *minganense* from Canada (UC, 1333117), showing smaller ornamentations than those of normal ones of var. *lunaria*. N. *B. matricariaefolium* from Michigan, U. S. A. (KYO), showing small verruca-like projections. O. *B. pumicola* from Oregon, U. S. A. (TOHO), showing small and dense verruca-like projections.

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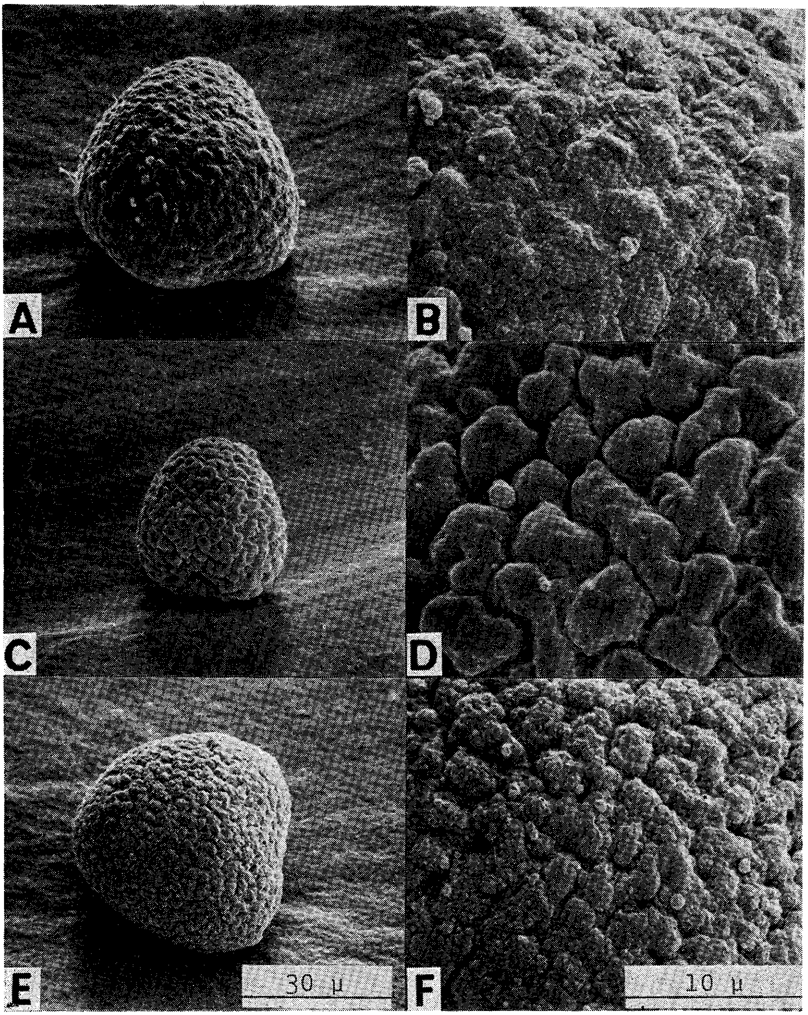
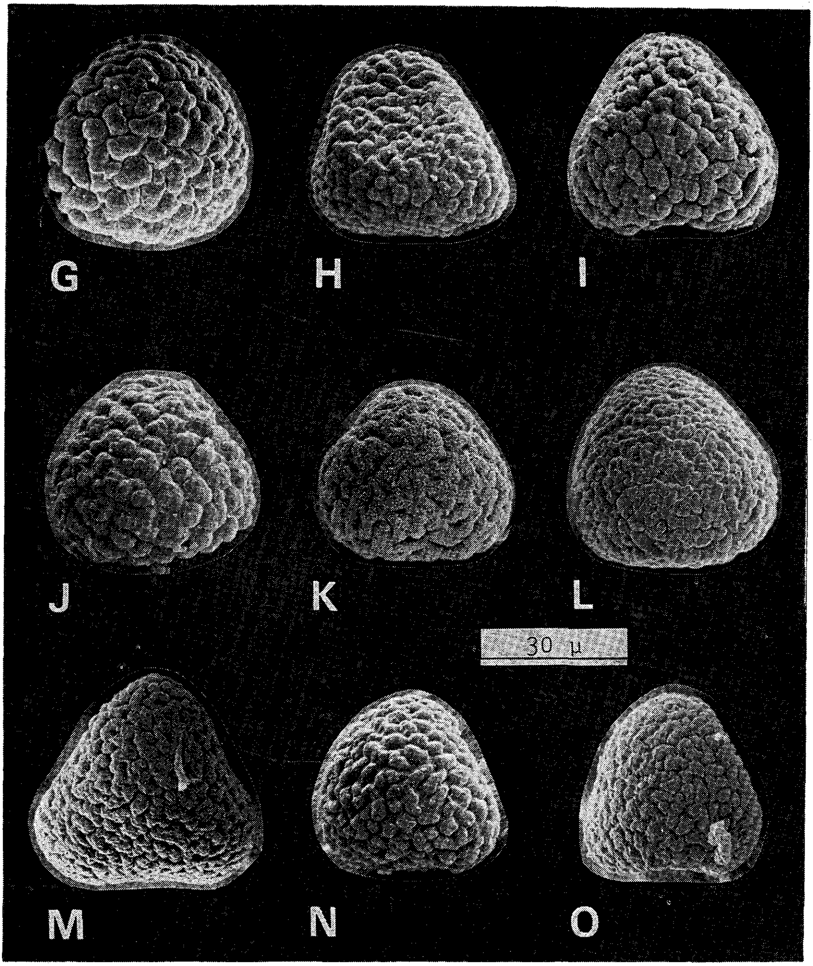
西田 (1952) の分類による *Botrychium* ヒメハナワラビ属，あるいは Clausen (1938) の Eubotrychium 亜属に属する6種の胞子の形態を光学顕微鏡と走査型電子顕微鏡で観察した。Clausen は同亜属を Sect. Lunariae のヒメハナワラビ節と Sect. Lanceolatae ミヤマハナワラビ節の二つに分け，前者に5種後者に1種を配したが，今回筆者は前者の Sect. Lunariae の中に二つのグループを認めた。その根拠は *B. simplex* の胞子が他のものに比べ大きさや彫紋が明らかに異なるためである。一方後者の Sect. Lanceolatae は胞子形態学上からは，特有の彫紋を有し Sect. Lunariae との区別は比較的容易である。したがって西田の *Botrychium* は胞子の形態学からは三つのグループに分けるのが妥当と考える。しかし個々の種の区別は容易ではない。また今回は日本と外国産の同じ種の胞子の形態学的比較を行なった。その結果の一つとして，八ケ岳で採集されたコケハナワラビ (西田) は *B. simplex* var. *tenebrosum* ではなく，*B. lunaria* var. *lunaria* ヒメハナワラビの矮小形であることがわかった。

□ J.S. Womersley: **Handbooks of the Flora of Papua New Guinea** vol. 1. pp. 278. ff. 115. 1978. Melbourne Univ. Press. 8,840円. パプア・ニューギニアのフロラの第一巻である。この地方は世界有数の種類の豊富なところとされながら，報告は出てもまとまったフロラとしてはなかっただけに今後が期待される。今まで3ケ国に領有され，それに引きつづいての委任統治，独立と歴史を短時日で経て来ただけに，その呼び名までまことに分かりにくかった。それを一応政治から脱脚して，Western New Guinea (6地方), North Eastern New Guinea (6地方), Papua (7地方), Bismarck Archipelago (3地方), Solomon Islands (8地方) とに大別し，これを一括して Papuasia と呼ぶことを主張している。

はじめにフロラを low land, montane, subalpine とに分け，前者を海岸，湿地，低地雨林，常緑林，サバナ，低木林及び草地に，中者を 1000〜3800 m とし，イネ科の湿地と山岳降雨林を上中下に，それ以上の亜高山帯とにわけて述べている。4200 m ほどの高山ではキンポウゲ，リンドウ，イヌノヒゲ，キジムシロ，ソモソモの類と，大部分が日本とひどく似ているのが面白い。この意味ではアジア的でさえある。その次に11科だけ各科毎に科の記載，検索表，各属と種をあげ，適当に図を加えて論じてある。ヒエ科，タデ科は別としてほとんどが標本から描かれた図で，記述もそれに準ずるだろうから，同定は大変であろう。*Terminalia* 各種の果実はじつに多型であるのに驚く。Datiscaceae の2属2種も興味がある。全部でパプアシアには209科あるというから，まだその5%で，それも少数種の科だから，今後の発展を期待したい。　(前川文夫)



N. SAHASHI: Ophioglosales in Japan



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